AN HISTOPATHOLOGIC STUDY OF THE EFFECTS OF A SUBSTITUTED PHENANTHRENE DERIVATIVE ON SARCOMA 37 IN MICE

by

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INTRODUCTION

Cancer chemotherapy dates back almost to the beginning of the history of medicine. Its systematic and substantial development, however, had to await the recognition of the cellular nature of neoplasms, the growth of experimental oncology and the general principles of experimental therapeutics. Less than six decades have elapsed since the most recent of these events and in this period thousands of chemicals, both synthetic and natural materials, have been tested in dozens of ways against different experimental tumors. Although these efforts have so far yielded a small number of agents of proven clinical value against cancer in man, they have led to the accumulation of an extensive body of information which has contributed significantly to our knowledge in cancer research.

The present study, devoted to the preclinical screening of a newly synthesized, substituted phenanthrene derivative against Sarcoma 37 in mice, is an attempt to explore the possibility of finding an agent of clinical value. In the following pages a study on the histopathologic changes associated with the effects of 3,6-diacetate of 9,10-diethylphenanthrene on Sarcoma 37 is described. For purposes of comparison, the parent compound, diethylstilbestrol diacetate, was included in the study.

REVIEW OF LITERATURE

According to a recent review by Plattner (1964) it was about 100 years ago that Lissauer (1865) first used potassium arsenite in the treatment of two cases of leukemia. A long hiatus ensued during which many drugs were tried, but nothing of importance was found. In the early 1940's estrogens were used in metastatic prostatic cancer, and both sex hormones were given

to patients with advanced breast cancer. The above author (Plattner, 1964) indicated that a clinical report on urethane appeared in 1946, but after a period of use in chronic leukemias and multiple myelomas it had been largely superceded by other drugs. The author further stated that a major landmark was the first report on the polyfunctional alkylating agents presented by C. P. Rhoads shortly after the end of World War II. Since then a number of compounds, related to nitrogen mustard, which have proved more acceptable in certain situations have been described; these include triethylenemelamine, chlorambucil, triethylene thiophosphoramide, busulfan, and cyclophosphamide. In rapid succession the folic acid antagonists, the adrenal corticosteroids, and 6-mercaptopurine became available, and the accelerated pace of discovery heralded a bright future.

Plattner (1964) stated that since 1954 the major drugs which have been found to be of practical value include 5-fluorouracil, O,P-DDD (1,1-dichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl)ethane), and the vinca alkaloids, vinblastine and vincristine. Compounds of unproved value include the colchicine analogues, mitomycin C, methylglyoxalbis (guanylhydrazone, methyl-GAG), hydroxyurea, methyl hydrazine (N-isopropyl-a-(2-methyl hydrazino)-p-toluamide), cystosine arabinoside and quinacrine.

Plattner also noted that cancer chemotherapy at present has come to fall in the following four broad groups of compounds: alkylating substances, antimetabolites, antibiotics and alkaloids, and hormones and miscellaneous compounds.

Hormone Therapy

Huggins et al. (1941) observed that hormones are significant factors in

the etiology of cancer, its prevention and its treatment. The treatment of mammary cancer by endocrine methods rests on the concept of hormonal dependence, which characterizes many of these cancers. It has been learned that the growth of several cancers, including certain carcinomas of the breast, can be stimulated or inhibited by the administration or withdrawal, respectively, of appropriate endocrine substances. Beatson in 1896 made a highly important discovery when he found that removal of ovaries caused regression of breast cancer in women. This classic work was done before there was any concept of hormones. Many hormonal methods, since then, have been employed for the treatment of breast cancer and prostatic cancer.

Estrogens, originally regarded as important factors in the development and growth of breast cancer, were first demonstrated by Badger et al. (1942) to have an inhibitory effect on established lesions as well. This challenged the earlier concepts and provided impetus to a more thorough investigation. Nathanson (1944) reviewed the clinical evidence relating the incidence of cancer of the breast to age, early castration, and menopause and discussed the first attempts with hormone therapy. At about that time the first data on estrogen therapy of inoperable carcinoma of the breast appeared. Since then large series have accumulated. Haddow et al. (1944) demonstrated the regression of prostatic cancer after administration of estrogens. The authors had also administered phenolic estrogens to a series of patients with advanced cancer; the estrogens were triphenylchloroethylene, triphenylmethylethylene and stilbestrol. Nathanson (1946) stated that since then a considerable number of natural and synthetic estrogenic preparations have been utilized and by far the largest experience has been with diethylstilbestrol; but comparable results have also been obtained with most of the

other preparations.

Contrary to the expectations of hormonal therapy, Huggins et al. (1941) for the first time noted that the patients who had received estrogen treatment for eight to 23 days against prostatic cancer, developed small, palpable areas of subareolar breast tissue. Nathanson (1946) described the effect of stilbestrol on advanced cancer of the breast. He stated that stilbestrol may exert an initial beneficial but temporary effect. The effect on metastases was open to question. The favorable response was confined to older women and the mechanism of action was not clearly understood. Taylor et al. (1948) observed that the only clearly determined factor influencing the response of the patients with breast cancer was age. Persons of advanced age responded the best. Emerson et al. (1949) made histological studies on biopsies and whole breast specimens from patients with breast cancer treated with estrogens. The consistent changes were replacement of tumor tissue by fibrous tissue. In the early stages of regression necrosis of tumor cells accompanied fibrous proliferation. Herrmann et al. (1949), in the course of therapy with diethylstilbestrol, observed reactions associated with hypercalcemia and renal insufficiency in patients with breast cancer.

Stilbestrol has been the estrogenic preparation most widely used in the treatment of prostatic cancer. Nathanson and Kelley (1952) indicated that there was much disagreement about optimal dosage of the estrogens in the treatment of prostatic cancer. The authors described the side effects of estrogen administration in the male as loss of sexual power, shrinkage of the penis, excessive weight gain and painful enlargement of the male breast even to the extent of bilateral cancer. In some cases treatment had to be abandoned because of the intolerable side effects. The authors also described

the side effects of estrogen administration in the treatment of breast cancer. They observed enlargement of the breasts, tenderness of the nipples, deep pigmentation of the nipple and areola, uterine bleeding in patients over sixty which was frequent but not severe and incontinence, which was one of the most distressing symptoms. Kennedy et al. (1953) observed that hypercalcemia was a potential complication during estrogenic hormone therapy of patients with advanced breast cancer.

Hormone therapy with estrogens was gradually discontinued in the following years because of the undesired side effects. In search of compounds which may be inhibitors of cancer, it thus became essential that the compound should have no or very little estrogenic activity. Knoche and Gawienowski (1962) proposed investigation of 3,6-derivatives of 9,10-diethylphenanthrene derived from diethylstilbestrol by ultraviolet irradiation. The authors had made a study on these compounds which indicated that 3,4,5,6,12,13-hexahydro-3,6-dioxo-9,10-diethylphenanthrene (DEP-DK) and 3,6-dihydroxy-9,10-diethylphenanthrene (DEP-DH) had 1/150th of estrogenic activity of diethylstilbestrol. The present study was based on this investigation to determine the effect of a related phenanthrene derivative obtained by ultraviolet irradiation of diethylstilbestrol on mouse tumor transplants of Sarcoma 37 and also its estrogenic activity, if any.

The Effect of Hormones on Mouse Tumors

Gardner (1939) observed that intrinsic factors other than estrogens played a significant role in mammary carcinogenesis in mice, and in some strains of mice these intrinsic factors were largely dominated by the effects of estrogens administered for long periods. Bittner (1941) indicated that

the administration of estrogenic hormones often resulted in the induction of mammary tumors in mice which had an inherited susceptibility for spontaneous tumors and an active milk influence. He showed that tumors were not induced in any animal which had been nursed by females of strains which had low incidence of spontaneous tumors, and suggested that by foster nursing it should be possible to determine if the influence in the milk was active or inactive in mice of any strain. The above author also observed that foster nursing did not influence the genetic susceptibility of an individual for the development of spontaneous mammary tumors. Allen et al. (1941) observed that after extended treatment with high doses of estrogen, carcinomas of the uterine cervix had appeared in 15 of 24 mice. He further showed that genetic factors involved in mammary cancer seemed to have little influence as determining factors in cancer of the cervix uteri.

Gardner (1941) found that tumors of the adrenal glands arose in 13 of 15 ovariectomized mice of an early generation of the NH strain. These adrenal tumors were associated with evidence of estrogenic stimulation as determined by the condition of the uteri and mammary glands. The uteri showed cystic glandular hyperplasia and the vaginal epithelium was thickly stratified although not cornified. Jones (1941) observed that injections of testosterone propionate in virgin female mice of C_3H strains from the second to twelfth month of life lowered the incidence of spontaneous mammary gland tumors but did not inhibit growth of mammary gland tumors once they had reached macroscopic size. Bittner (1942) determined that for the development of mammary carcinomas in virgin and breeding females three factors had to be present and active: an active mammary tumor milk influence which was generally transferred by nursing; an inherited susceptibility to the development

of spontaneous mammary tumors; and a hormonal stimulation of the mammary tissue resulting in growth suitable for the cancerous change. He further demonstrated that the mice with high incidence were the C_3H and A strains; the strains with low incidence were the C57 and C strains of mice. The above author further indicated that the administration of estrogenic hormones produced mammary tumors in males and females from strains of mice with the active milk influence. The induced incidence was usually higher in females than in males and the stimulation produced by large amounts of estrogenic hormones could overcome the threshold of genetic nonsusceptibility in the presence of an active milk influence.

Heiman (1943) observed that progesterone inhibited the growth of the adenomatous portion of spontaneous rat mammary fibroadenoma. Shrinkage was followed by fibrosis. He also indicated that 18 mg. of progesterone did not interfere with the stimulating effect of 2.5 mg. of estrogen. Comparative studies were made by Howard et al. (1944) on the effectiveness in inhibiting the growth of implanted sarcoma 180 by the use of three hormones; estrone, diethylstilbestrol and progesterone. In certain strains estrone was found effective both in males and females whereas diethylstilbestrol was effective in females only. In certain substrains having a high incidence of spontaneous tumor these estrogens were not effective unlike the low incidence substrains which showed beneficial results from the administration of estrogen. The author emphasized that the milk factor was involved in resistance to tumor growth.

The action of diethylstilbestrol was investigated by Ludford et al. (1947) on ten different transplantable mouse tumors in mice of four inbred strains. On these tumors stilbestrol showed no specific inhibition of growth.

The author emphasized that the genetic constitution was a significant factor in determining sensitivity to the toxic action of stilbestrol. Pan et al. (1948) observed that 4 of 10 estrogen-treated hybrid mice (C_3H females X PM males) that tolerated the treatment more than 200 days had carcinomas of the uterine cervix or vagina.

Vaginal assays of estrogenic activity were performed by Trentin (1950) on groups of 20 mice. Graded doses of estrogen were given. The minimum dose of estradiol benzoate giving full cornification in approximately 50% of the animals varied from 0.06 to 0.27 micrograms. The author showed that the presence or absence of milk factor, obtained by foster nursing did not alter vaginal sensitivity to estrogen.

Bittner (1952) presented experimental data on the transmission of a spontaneous mammary tumor by males to females, which usually was considered to be transferred exclusively by females in the milk. He showed that the infected female could become cancerous or remain noncancerous, but that they transferred the agent in their milk to their progeny which then had a high incidence of mammary tumor. The agent for mammary cancer was recovered from the mammary tumors that developed in either infected females or their progeny.

According to Shapiro (1952), the beneficial response of certain human malignancies to stilbestrol therapy had warranted numerous studies to find a laboratory neoplasm which would similarly respond. Most of them were unsuccessful, until Sarcoma 180 was utilized. The author found that diethylstilbestrol inhibited the growth of the 755 mammary adenocarcinoma in C57BL mice and the combination of 8-azaguanine and stilbestrol had a striking carcinostatic effect upon this tumor. This same author had shown that the

weight loss induced by stilbestrol in the experimental animals was reduced to insignificant levels by addition of testosterone without affecting carcinostatic activity. He further emphasized that a proper genetic background was the only factor responsible for the effectiveness of stilbestrol in mice.

Bilateral intraocular transplants of an adrenal cortical tumor from a CE mouse were made by Browning et al. (1959) in intact and castrated male and female CE/BALB/C hybrids. Various experimental groups were treated with progesterone, estrogen and testosterone. In castrated males progesterone accelerated growth, estrogen moderately slowed growth, but estrogen with progesterone markedly slowed growth, and testosterone completely inhibited it.

The effects of estrogens were studied by Angrist et al. (1960) with and without foreign body on squamous metaplasia of the bladder of rats maintained on a low vitamin A diet. Estradiol increased the degree of metaplasia in the bladder when combined with vitamin A deficiency and/or foreign body stimulation. Svaboda (1961) observed the effect of long term absorption of stilbestrol on the incidence of skin tumors induced by 3-methylcholanthrene and the neutral fraction of tobacco tar. The incidence of the carcinoma in the methylcholanthrene-plus-stilbestrol group was 39%, whereas in the methylcholanthrene-only group the incidence was 17%. Mammary tumors were induced by Sternatal et al. (1963) with 20 mg. of 7,12-dimethylbenzanthracene. Subsequent adrenalectomy-ovariectomy and hypophysectomy performed within 10 to 20 days after tumors were first detected resulted in tumor regression in all the animals. Estrogen administration reactivated tumor growth after adrenalectomy-ovariectomy but not after hypophysectomy showing the dependency of estrogen stimulation upon presence of the pituitary gland.

Sarcoma 37

The original tumor from which Sarcoma 37 was derived was a spontaneous adenocarcinoma, designated Carcinoma 37, that appeared in the thoracic region of an old female mouse of the Imperial Cancer Research Fund Stock. The tumor was resected on September 25, 1906, but recurred shortly thereafter, and the mouse was killed on November 22, 1906. The origin and early transplant generations of Carcinoma 37 and its transformation to an anaplastic tumor were described by Haaland (1908) and by Bashford (1911). The original tumor as described by Haaland was composed of acini and solid masses of cells with some of the acini containing secretory material. The stroma was delicate in some areas and abundant and cellular in others. The early generation transplant tumors were similarly composed chiefly of acini and solid masses of cells in varying proportion, but some showed cystadenomatous structures lined with papillary excrescences. Forty to 50 percent of the transplants grew successfully, beginning with the second transplant generation. Some sublines of subsequent generations continued to reproduce the pattern of carcinoma; others eventually came to resemble a sarcoma. This sarcomatous transformation occurred several times in descendants of a series of transplanted tumors. The transformation from carcinoma to sarcoma was a gradual process in transplants propagated over a period of 5 to 18 months after the initial transplantation of Carcinoma 37. Once the sarcomatous pattern was firmly established in all parts of the transplantable tumor, the growth did not revert to a carcinoma in any instance. Haaland (1908) in describing the transformation to sarcoma observed that the connective tissue stroma became more cellular and increased in quantity. Broad bands of atypical spindle-shaped cells appeared in the stroma, and these in turn were replaced by atypical

polymorphous cells considered to be the sarcomatous component. Necrosis of the epithelial portion of the tumors appeared more frequently. With the introduction of the sarcomatous element, the tumors were interpreted as being of mixed type. Coincident with these changes in morphology, the growth rate was increased. Metastases found in the lungs and elsewhere were either carcinomatous, sarcomatous, or mixed in appearance. Eventually, in most instances the carcinomatous elements were entirely replaced by sarcoma. A tumor of one of the sublines that contained short, spindle-shaped cells for several generations finally became stabilized as a polymorphous-cell sarcoma. It was this tumor that later became known as Sarcoma 37. Although Sarcoma 37 grew successfully in 90 to 100 percent of hosts inoculated, it frequently regressed. The tumor grew rapidly, invaded tissues adjacent to it, and developed central necrosis as early as ten to fourteen days following transplantation. Metastases were found in the lymph nodes, lungs, liver, spleen, myocardium, and kidneys.

Although not strain specific, the transplant tumor has been observed to grow better in some strains of mice than in others. Leiter and Kline (1953) described their observations on the growth and regression of Sarcoma 37 in about 2000 mice of 12 strains. According to these authors, the tumor grew progressively without regression in more than 99 percent of the BALB/C, DBA/2, ABC, ZBC and (BALB xA)F_1 mice (formerly CAF_1) in which it was transplanted. Regression rates of 42 to 78 percent were observed in C_3H, C57BL, CF, CFW, NIH-B.S., and NIH-Reg. mice. The Sarcoma 37 transplants grew for a week or ten days before the signs of regression appeared.

According to Stewart et al. (1959) Sarcoma 37 has been used extensively to evaluate the effectiveness of chemotherapeutic agents. The tumor is

valuable for such work, due to its successful transplantability and its rapid growth in mice of many strains. Moreover, transplants become richly vascularized as early as five days after transplantation, and the untreated tumor shows little degeneration and necrosis within the first eight or ten days.

Peters et al. (1946) during the first screening in tumor bearing mice described the following compounds to have yielded microscopic evidence of damage to Sarcoma 37: Cephalin hydrochloride; carydine hydrochloride; 1-(p-methoxyphenacyl)-3-picolinium iodide; 2-phenacylisoquinolium iodide; proflavine and d-tubocurarine chloride. The microscopic evaluation was based on mitotic activity of the tumor cells and necrosis. Diller (1947) observed that 0.01 mg. of Serratia marcescens polysaccharide in a volume of 0.1 ml. of sterile saline when given intraperitoneally produced nuclear damage to transplanted mouse Sarcoma 37 cells. Maximum destruction was attained at six hours. Mitotic division of tumor cells was inhibited for three days following treatment. MacCardle and Downing (1947) found that N-acetyliodocolchinol methyl ether attacked Sarcoma 37 by arresting mitoses; necrosis followed. Another compound, podophyllin, was described as having induced cell damage throughout the tumor with marked hemostasis and vascular damage.

In Swiss mice bearing Sarcoma 37 transplants, it was demonstrated by Creech et al. (1948) that passive immunization with the antibody containing fractions toward the P-3 type polysaccharide from the G. W. strain of Serratia marcescens, prior to the administration of relatively large doses of that polysaccharide, definitely decreased the mortality rate without interfering significantly with the tumor-necrotizing action of the polysaccharide. Belkin et al. (1949) reported the effect of a single intraperitoneal injection of one ml. of a preparation containing about 285 million

lysed Trypanosoma cruzi organisms and the effect of six such daily intraperitoneal injections on Sarcoma 37. No gross evidence of any effect was observed. Histologic studies revealed no tumor damage. Leiter et al. (1949) found that a number of compounds structurally related to podophyllotoxin showed little or no activity against Sarcoma 37. Leiter et al. (1950) later observed that podophyllotoxin, alpha-peltatin, and beta-peltatin were highly potent against Sarcoma 37. The minimum effective dose for a single subcutaneous injection was two micrograms per gram of body weight and the tumor damage was observed grossly six hours after injection.

Finkelstein et al. (1951) reported that multiple doses of 8-azaguanine produced a definite inhibition of growth and extreme cellular damage of Sarcoma 37. Gross tumor damage was noted within two to six hours, with extensive morphological damage occurring 24 hours after a single large dose of 8-azaguanine. These authors also observed that the drug was not completely destructive to this tumor as typical morphology of the tumor was seen on cessation of therapy. Kidder et al. (1951) observed that Sarcoma 37 in C57 black mice was not inhibited by the administration of 8-azaguanine.

The finding that methylation of the seven-membered C-ring in colchicine generally conferred higher potency against Sarcoma 37 in comparison with the unmethylated analogs was reported by Leiter et al. (1952). These authors also observed that the methylated compounds were less potent than the unmethylated when the C-ring was a six-membered aromatized one. Ninety-two arsenicals were examined in over 3000 tumor bearing mice by Leiter et al. (1952) for potency in damaging Sarcoma 37 following a single subcutaneous injection. The compounds were aliphatic and aromatic derivatives containing pentavalent or trivalent arsenic. A dose near maximum tolerated was required

for production of tumor damage with all of the active compounds. In investigations with the 14 amide derivatives of colchicine, it was found by Leiter et al. (1952) that colchicineamide was the only compound which gave a ratio for maximum tolerated dose per minimal effective dose considerable greater than that of colchicine (approximately 20 and 2 respectively).

Sixty-nine compounds were examined by Leiter et al. (1953) for potency in damaging Sarcoma 37 following a single subcutaneous injection in CAF_1 mice. They included 30 biphenyl, 11 tropolone and 28 phenanthrene derivatives and analogs. A dose near or above the maximum tolerated was required to produce damage. The iso-forms of four potent colchicine derivatives, colchicine, colchicine ethyl ether, colchicinamide, and trimethyl colchicine acid methyl ether-d-tartrate, were all found to be inactive when tested in an identical fashion by the same authors. These authors also emphasized the importance not only of the nature of the substituents, but also of their position in the C-ring of the colchicine molecule.

That 17 out of 27 derivatives of 1,3-diphenylpropylamine induced damage in Sarcoma 37 following a single subcutaneous injection was observed by Leiter et al. (1953). Two of 10 derivatives of 2,3-diphenylpropylamine induced damage in the tumors, while 34 diphenylpropylenediamines and 26 diphenyl alkanes were found to be inactive in producing tumor damage. The effect of various hexoses upon Sarcoma 37 growing in tissue culture was investigated by Rubin et al. (1954). Of the substances tested, a one percent equimolecular mixture of D-glucose and ammonium chloride proved to be the most toxic for tumor tissue. Leiter et al. (1955) recorded negative results with diphenylethylamines in producing damage in Sarcoma 37. Pradhan et al. (1956) investigated the tumor-necrotizing effect of Serratia marcescens

polysaccharide, pitressin, serotonin, amphetamine, histamine and acetyl-podophyllotoxin-2-pyridinium chloride on Sarcoma 37. These authors observed that the tumor damaging effect of nearly all the above drugs was reduced by the administration of cortisone, atropine, dibenamine, urethane or pentobarbital sodium. However the tumor-necrotizing effect of acetyl-podophyllotoxin-2-pyridinium chloride was not impaired by any of the above drugs.

Injection of mice bearing transplanted Sarcoma 37 with living and killed Candida species caused sloughing of the tumors; this was observed by Mankowski et al. (1957). The authors found that following intravenous injection of Candida guilliermondi there was widespread distribution of the organisms in normal tissues, as well as in tumors; no evidence of destruction in cells other than those of the tumor was noted.

Havas et al. (1958) noted that the toxins obtained from several strains of Streptococcus pyogenes and Serratia marcescens caused regression of Sarcoma 37. The combined preparations caused higher regression rates than streptococci alone. Serratia marcescens alone could also cause regression of the tumor. Donnelly et al. (1958) reported tumor regression of Sarcoma 37 with mixed bacterial toxins. They described microscopic changes in the tumor consisting of mitotic inhibition, pyknosis of nuclei, shrinkage of cells, and cellular disintegration with hemorrhage. Grossly, hemorrhage and eventual sloughing of the tumor were followed by healing and scar formation. It was concluded that bacterial toxins brought about tumor necrosis.

The polysaccharides obtained from higher plants induced hemorrhage and necrosis in Sarcoma 37 as observed by Belkin et al. (1959). Some of the plant polysaccharides that showed evidence of tumor damage were extracted from burdock root, wild bryony root and meadow safron root. Diller et al.

(1960) investigated the tumor damaging activity of the yeast polysaccharides on Sarcoma 37. It was noted that hydroglucan had a greater oncolytic activity than zymosan. Diller et al. (1963) subsequently found that one mg. of hydroglucan, when given intravenously, caused regression of 90 to 95 percent of Sarcoma 37 in C_3H mice. Lower percentages of regression of the tumor followed administration of the same amounts of hydroglucan by the intraperitoneal, intramuscular, and subcutaneous routes.

Hirschberg (1963), in an exhaustive review of anticancer agents, reported that of 79 compounds that were tested against Sarcoma 37 up to 1958, 55 had an inhibitory effect. These compounds included various groups of nitrogen mustards, colchicine derivatives, steroids, ethylamines, purines and miscellaneous hormones.

Phenanthrene Substitutes

One general difference between the physiology of normal and malignant tissues as described by Furth (1953) is that tumor tissue is ordinarily faster growing than normal tissue; there are proportionately more cells in the process of division in the cancerous tissues. An attempt was made by different investigators to utilize this property therapeutically. Dustin (1934) observed that colchicine affects mitosis both in animal and vegetable tissues. Brues et al. (1937) found that colchicine inhibits mitoses of the tumor cells in the metaphase. This property of colchicine was exploited in further investigation. It is well known that colchicine contains a phenanthrene nucleus.

Turner (1939) investigated empirically compounds whose only apparent relationship to colchicine was a phenanthrene nucleus with various

substituents at different positions. The author tested 75 phenanthrene derivatives in 1841 mice bearing transplanted sarcomas and 62 mice bearing spontaneous carcinomas. No regression occurred in the mice having spontaneous tumors but there was tumor regression in from one to 50 percent in mice bearing transplanted sarcomas. Of the 75 phenanthrene variants, dihydrothebainone hydrochloride, dihydro-des-N-methyldihydrothebainone, 9-aminomethyl phenanthrene hydrochloride, dihydrothebainone, des-N-methyldihydrothebainone, 3[2-(diethylamino)-1-oxoethyl] phenanthrene hydrochloride and 3-(2-piperidino-1-oxoethyl) phenanthrene hydrochloride were found to be the most effective. This same worker, however, stated that the tumor damaging activity could not be satisfactorily associated with any specific chemical grouping. Turner later (1943) tested a series of 43 phenanthrene derivatives and observed that 9-(2-methylamino-1-oxopropyl)-phenanthrene hydrochloride, when given intraperitoneally in 74 doses of 2.5 mg. each, caused regression of mammary carcinoma of spontaneous origin in Swiss mice. The percentage of regression was not mentioned by the author.

Twenty-eight phenanthrene derivatives and analogs were examined by Leiter et al. (1953) for potency in damaging Sarcoma 37 in CAF_1 mice. A dose near or above the maximum tolerated dose was found to produce damage. Of the 28 phenanthrene derivatives, 1-methyl-5-(9-phenanthryl)-biguanide HCl, phenanthraquinone, o-, m-, and p-phenanthroline were found to be active. Of the above five compounds, the first two were true phenanthrene derivatives and the remaining three compounds were analogs of phenanthrene in which two heterocyclic rings replaced benzene rings. Of these only o-phenanthroline was able to damage Sarcoma 37 at a dose of 30 micrograms per gm. body weight which was appreciably below the maximum tolerated dose (80-100 micrograms

per gm. body weight).

Turner (1958), in subsequent studies on phenanthrene compounds, found the following six compounds caused regression in Sarcoma 37: 2,9-acetyl-phenanthrene; 3-(2-amino-1-hydroxy-N-propyl) phenanthrene hydrochloride; 9-aminomethyl phenanthrene hydrochloride; 2-(3-diethyl amino)-1-oxopropyl)-phenanthrene; ethoxy-4-acetylamino phenanthrene; and 3-(2-ethylamino)-1-oxopropyl) phenanthrene hydrochloride. Of these six compounds it was indicated that ethoxy-4-acetylamino phenanthrene had the maximum activity. Five mg. of the compound given in 13 doses in mice with Sarcoma 37 caused tumor regression. The ratio between the sizes of the tumor in treated mice and the controls was 15.

Field et al. (1959) made an extensive study by testing 170 phenanthrene derivatives for tumor-inhibitory effect on another mouse tumor, Sarcoma 180. Eight of the compounds tested were observed to inhibit the growth of Sarcoma 180. The resulting tumors were significantly smaller than control tumors (25 percent or more). The authors noted that no hydroaromatic phenanthrene derivative was active nor was any derivative which carried a substituent in the 2-position. All active compounds had substituents either in the 3-position or meso-position, often in both. It was also observed that all active phenanthrene derivatives were amines, in most instances tertiary amines. Among the tertiary amines there appeared to be an optimum chain length of five to eight carbon atoms in the alkyl groups attached to nitrogen. It was also shown by the same investigators that no phenanthrene derivative could induce reduction in tumor size greater than 35 percent. Histopathologic studies revealed a decrease in mitotic figures and increased presence of areas of necrosis. Pyknosis, karyorrhexis and karyolysis were observed in

the tumor cells. The following phenanthrene derivatives were found to be active in the above study: 1-(9-phenanthrylmethyl amino)-2-propanol; 9-bromo-3-(3-diamylamino-1-oxopropyl)-phenanthrene; 9-chloro-3-(2-dihexylamino-1-hydroxyethyl)-phenanthrene; 3-(2-diamylamino-1-hydroxyethyl)-phenanthrene; 9-chloro-3-(2-dioctylamino-1-hydroxyethyl)-phenanthrene; 9-chloro-3-(2-diamylamino-1-hydroxyethyl)-phenanthrene; 3-(2-amino-1-chloroethyl)-phenanthrene; and 9-bromo-3-(3-dihexylamino-1-oxopropyl)-phenanthrene. Tumor regression with the above compounds ranged from 28.5 to 35 percent. These authors stated that five of the eight phenanthrene derivatives were beta-amino alcohols and two were amino ketones. The most active compound was 3-(2-amino-1-chlorethyl)-phenanthrene hydrochloride, a beta-amino chloride with which there was a maximum regression of 35 percent. It was indicated that the enhanced activity of this compound was due to the analogy between this compound and the nitrogen mustards. As in the case of nitrogen mustards, replacement of the chlorine in the above compound by an unreactive group resulted in loss of biological activity; for example, it was shown that 3-(2-amino-1-methoxyethyl)-phenanthrene hydrochloride was found to be inactive against Sarcoma 180.

Knoche and Gawienowski (1962), in a search for compounds which inhibit cancer yet have very low estrogenic activity proposed investigation of 3,6-derivatives of 9,10-diethylphenanthrene derived from diethylstilbestrol and its derivatives by ultraviolet irradiation. They also observed that 3,4,5,6,12,13-hexahydro-3,6-dioxo-9,10-diethylphenanthrene and 3,6-dihydroxy-9,10-diethylphenanthrene had minimal estrogenic activity compared to the diethylstilbestrol.

MATERIALS AND METHODS

Compounds Studied

Studies were made in collaboration with Sister Mary Grace Waring, Ph.D., Marymount College, Salina, Kansas, who prepared the compound and undertook the toxicity studies and tumor transplantation. The compound, 3,6-diacetate of 9,10-diethylphenanthrene (DEP-DA), was prepared by a method originally suggested by Banes (1961) and Hugelshofer (1960); it involved irradiation of diethylstilbestrol by ultraviolet light to obtain the above mentioned phenanthrene derivative.

For purposes of comparison, the 4,4-diacetate salt of the parent compound, diethylstilbestrol (DES-DA) was also studied.

Tumor-susceptible C_3H/Hej Mice

Preliminary toxicity determinations and standardization of procedures were performed on three to four week old, non-pedigreed white mice.

Mice of the tumor-susceptible C_3H/Hej strain were obtained from the Jackson Laboratory, Bar Harbor, Maine. The offspring were used to maintain the strain, for toxicity and tumor inhibition studies. Arrangements were also made with the Jackson Laboratory to supply C_3H/Hej randomized mice when needed.

Sarcoma 37

Sarcoma 37 tumor was obtained from the Jackson Laboratory, Bar Harbor, Maine, in DBA/LJ mice and immediately transferred to the C_3H/Hej mice. Transfer was accomplished by preparing a brei of the excised tumor material in

Tyrode's solution (Harper, 1957) and injecting 0.2 ml. of this cell suspension subcutaneously in the right axillary region of the tumor susceptible mice.

Determination of the Toxicity of the DEP-DA and DES-DA

The toxicity studies were deemed necessary since there were no data available on the newly-synthesized compound. Therefore, the directions given in protocol 4, "Selection of dosages," Cancer Chemotherapy Report No. 25, December, 1962, were followed in this experiment.

Preliminary studies were made by injecting into mice 0.2 ml. of the vehicle in which the drug would later be administered in the toxicity studies. The carriers used were corn oil and olive oil. Mice were divided into the following groups.

Group A. Corn oil injection: Three male and five female C_3H/Hej mice were injected and an identical group was retained as controls.

Group B. Olive oil injection: Four male and four female C_3H/Hej mice were injected and an identical group was kept as controls.

Group C. Normal saline: Three male and three female C_3H/Hej mice were injected and four male and four female mice were utilized as controls.

Before injecting, the mice were shaved at the site of injection. The area was wiped with a cotton swab moistened with lysol, then with 70 percent alcohol. The injection was given subcutaneously in the thigh over the biceps femoris muscle. After one week each mouse was anesthetized with ether. A midline thoraco-abdominal incision was made; the area over the injection-site was also incised to indicate where the injection was made. The brain

was removed. The mouse and its brain were placed in a square of gauze, tagged, and placed in 10% buffered formalin.

Only C_3H/Hej female mice were used in toxicity studies on DEP-DA and DES-DA. Injections, which were carried out at intervals of 0, 48, 72, and 96 hr, were made on the back of each mouse in such a way that the four points of injection would make a square. Dosage was determined by calculating the average weight of each group. Mice were sacrificed on the seventh day after the last injection.

Group 1: Injection of DEP-DA in 0.2 ml. of corn oil was given at a rate of 46.6 mg./kg. mouse. The mice were 40-41 days old. Four mice were used.

Group 2: Injection of DEP-DA in 0.2 ml. of corn oil was given at a rate of 96.5 mg./kg. mouse. Mice were 39 days old. Four mice were used.

Group 3: Injection of DES-DA in 0.2 ml. of corn oil was given at a rate of 45.8 mg./kg. Mice were 40-41 days old. Four mice were used.

Group 4: Injection of 0.2 ml. of corn oil only was given per mouse. Four mice were used.

Studies with DEP-DA and DES-DA on Tumor Transplants

Tumor material was excised from donor mice and used to prepare a brei in Tyrode's solution. Eighty C_3H/Hej mice, 36 $\pm$ 3 days old, received 0.2 ml. of the cell suspension subcutaneously in the right axillary region. The mice in which the transplanted tumor material began to proliferate (77/80) were divided into five groups, and were weighed by cages in order to determine the appropriate dosage of the compounds to be tested. The DEP-DA and DES-DA were dissolved in corn oil for this study. Five daily injections of the

compounds were made, starting the third day after tumor transplantation.

The groups of mice and their dosages were as follows:

Group 1: Two-tenths of a ml. of a solution containing 33.5 mg./10 ml. of DEP-DA was given to each mouse. At this rate each animal received 45 mg./kg. There were 16 mice in this group.

Group 2: Two-tenths of a ml. of a solution containing 67.0 mg./10 ml. of DEP-DA was given to each mouse. At this rate every animal received 90 mg./kg. Sixteen mice made up this group.

Group 3: Two-tenths of a ml. of a solution containing 26.8 mg./10 ml. of the DES-DA was given. Each mouse received the drug at a rate of 36 mg./kg. The group contained 16 mice.

Group 4: This group of 15 mice received 0.2 ml. of corn oil only.

Group 5: These 14 mice received no injection other than the tumor.

Pathogenesis Study

Twenty-six mice of the C_3H/Hej strain were used in this study. Mice were weighed before injection of the tumor. Two-tenths of a ml. of tumor brei of Sarcoma 37 was then injected into each mouse. Five injections of DEP-DA at the higher dose, i.e., 90 mg./kg., were made starting on the fourth day post-tumor inoculation. Each day following tumor injection one mouse was sacrificed for study. The weight of the mouse and the tumor dimensions were recorded at the time of sacrifice. Tissues from these mice were collected as before for histopathologic examination.

Preparation of Tissues for Histopathologic Examination

The mice from the toxicity studies and tumor-inhibition studies were

observed carefully. They were sacrificed if they appeared to be at the point of death and those which died were removed from the cages as soon as possible. All mice were preserved in 10% buffered formalin as described in an earlier section. Mice from both the toxicity studies and the tumor-inhibition studies were later dissected and organs collected from them as follows:

1. Liver
2. Stomach with Pancreas
3. Anatomic divisions of small and large intestines
4. Spleen
5. Kidneys with the adrenal
6. Testes with epididymis
7. Ovary and uterus
8. Lungs
9. Heart
10. Brain
11. Leg muscle
12. Piece of rib cage
13. Urinary bladder
14. Entire section of skin and muscle surrounding point of injection
15. Piece of tumor tissue
16. Any other tissue in the body where lesions were found grossly.

The fixed tissues were trimmed, embedded, sectioned and stained with histologic stains. Hematoxylin and eosin were used on all tissues routinely; special stains including VonKossa's, Wilder's silver stain and periodic acid-Schiff stain were also used to demonstrate calcification, reticulum and mucopolysaccharides, respectively.

RESULTS

Sarcoma 37

The tumor consisted of a solid mass of cells (Plate IV, Fig. 1)* interspersed with small amounts of stroma. The tumor cells were round, slightly elongated or occasionally pleomorphic and were closely packed. The cytoplasm was basophilic and homogenous; the nuclei were round or oval. Mitotic figures were numerous in the viable portions of the tumor (Plate IV, Fig. 2). The tumor was quite vascular as represented by small blood vessels throughout the substance of the tumor. A small amount of hemorrhage was noted in some parts of the tumor. The host tissues, especially the subcutis and muscles of the intercostal spaces, were infiltrated by the tumor cells and showed a slight inflammatory reaction with proliferation of young fibroblasts and hyperemic blood vessels.

Metastases were observed in the lung (Plate V, Figs. 1 and 2) and the liver (Plate VI, Figs. 1 and 2). The former were considered to be hematogenous since several single and multiple cell emboli were seen in interstitial capillaries. In the latter case, metastasis occurred in one instance by simple extension since the lesion was observed to be contiguous with the primary tumor; the other instance appeared to be hematogenous.

The untreated tumor showed rapid growth with little degeneration and necrosis within the first eight or ten days. Later the tumor outgrew the vascular supply and became necrotic. Necrosis of the tumor cells was characterized by pyknosis, karyorrhexis and karyolysis. Calcification was also

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*This plate and all subsequent plates are found in the Appendix.

seen (Plate VII, Fig. 1) in the degenerated and necrotic areas. Hemorrhage was consistently observed in the necrotic areas of the tumor. In the viable portions of the tumor there was abundant reticulum (Plate VII, Fig. 2). The degenerated tumor tissue was devoid of reticular fibres. In a number of instances the tumor bearing mice had a splenomegaly with lymphoid hyperplasia.

Toxicity Studies

The carriers used for the administration of the phenanthrene derivatives in the toxicity study were corn oil and olive oil. In the group of mice which received corn oil the principal change noted was a granulomatous reaction occurring in the skin at the site of injection. However, this change was not consistent in all the mice. The olive oil in general, produced a lesser reaction, the injection site being characterized only by accumulations of the oil in large subcutaneous spaces, which could be seen both grossly and microscopically. Corn oil was chosen as the carrier for the drugs because of the enhanced solubility of DEP-DA and DES-DA in it.

The mice which received DEP-DA at a rate of 46.6 mg./kg. were found to have many tissue changes. The principal change was observed in the uterus and consisted of endometrial hyperplasia (Plate VIII, Figs. 1 and 2). The endometrium was thickened and showed an increased number of uterine glands, most of which contained secretion. The blood vessels were found to be hyperemic. There were no cystic changes. These findings were consistent in all the mice receiving the above compound at that rate. The wall of the urinary bladder was also thickened due to an increased depth of the lamina propria and submucosal layer. The epithelial lining of the urinary bladder was found to have a tendency towards squamous metaplasia (Plate IX, Fig. 1)

but this was not well enough defined to say definitely, even though thickening of the bladder wall was consistent with every mouse in this group. The thickening of the uterine horns and the urinary bladder, it should be noted, could be seen at gross examination in most of the cases.

Another organ in which pathologic changes were observed was the kidney. Coagulation necrosis of the tubules was predominant. The necrosis in most of the cases was confined to the cortical region. In the rest of the organ pyknosis was evident. The degenerative changes in the kidney did not elicit any inflammatory reaction although some amount of congestion was observed in almost every case. No visible lesions were associated with the adrenal.

The liver was grossly enlarged in almost all the mice which received DEP-DA. Microscopically there was both cloudy swelling and hydropic degeneration seen in the cord cells; passive congestion was also observed. An interesting finding on the hematoxylin and eosin stained sections of liver was the presence of numerous, spherical, eosinophilic, refractile bodies, located intracellularly for the most part, but occasionally seen extracellularly. The bodies were neither PAS positive nor did they give a positive result when examined under polarized light.

The lungs were invariably congested. It was presumed that this change was principally due to the effect of ether that was used to sacrifice the animals. In some instances the brains were congested; this was also presumed to be due to the method of euthanasia employed on these animals. No lesions were found in the gastrointestinal tract, pancreas, spleen, heart or skin.

In the second group of mice that received DEP-DA at a rate of 96.5 mg./kg. the uterus was the primary site of pathologic changes. Grossly the uterus was enlarged; microscopically, there was endometrial hyperplasia and

cystic changes were seen in the endometrial glands of some uterine sections. There was secretion in the uterine glands and the blood vessels were hyperemic. Another important change in the uterus was squamous metaplasia (Plate IX, Fig. 2). Although in all the cases endometrial hyperplasia was a consistent lesion, squamous metaplasia was seen in about 50 percent of the cases. The changes in the liver were similar to those described for the lower dosage of DEP-DA.

In the mice that received DES-DA at a rate of 45.8 mg./kg. the most significant change occurred in the uterus which was grossly enlarged. There was endometrial hyperplasia and also squamous metaplasia. Both the pathologic changes were consistent in the group. The wall of the urinary bladder was also thickened.

Experimental Study With the Injections of Tumor and Compound

The first group consisted of mice that received DEP-DA at a rate of 45 mg./kg. The principal microscopic changes observed were areas of hemorrhage and necrosis in the tumor. Mitotic figures were reduced in the viable areas of the tumor. Karyorrhexis, karyolysis and calcification were seen in the necrotic areas of the tumor. Fibroblast proliferation in the tumor was not marked. Grossly the tumor assumed a crateriform appearance with scab formation following the hemorrhage and necrosis (Plate I, Figs. 1 and 2) (Waring, unpublished data).

The lungs were found to be congested with proteinaceous fluid in some alveoli. In a few mice of this group metastases were seen in the lungs. The uterine wall was thickened due to endometrial hyperplasia but it was not as marked as that observed in the toxicity studies. The lamina propria and

submucosa of the urinary bladder was thickened. Coagulation necrosis was seen in the renal tubules; pyknosis was generalized but with the exception of a slight amount of congestion there was no other reaction. The liver was found to be congested. No visible lesion was found in the gastrointestinal tract, heart or pancreas. Lymphoid hyperplasia was observed in the spleen.

In the second group of mice which received DEP-DA at a rate of 90 mg./kg., the following pathologic changes were observed. Grossly, there was even more shrinking of the tumor tissue than had occurred at the lower dosage of DEP-DA (Plate II, Fig. 1). Microscopically, the primary change in the tumor was an obvious decrease in the number of mitotic figures. Many areas of hemorrhage and necrosis were seen, typified by karyorrhexis, karyolysis, and calcification. Fibroblast proliferation and compression of connective tissue around the tumor were also observed. No metastasis was seen in any of the examined organs. Congestion was noted in the lungs and liver. Coagulation necrosis was observed in the renal tubules; pyknosis was a predominant feature. The uterus was grossly enlarged; microscopically there was endometrial hyperplasia. Unlike the toxicity studies, squamous metaplasia was not seen in a single instance; however endometrial hyperplasia remained a consistent lesion. Similarly the wall of the urinary bladder was thickened. No lesions were found in the brain, gastrointestinal tract, pancreas, or heart.

The mice in group 3 received DES-DA at the rate of 36 mg./kg. Changes comparable to those observed with DEP-DA occurred grossly (Plate II, Fig. 2). Necrotic areas were seen in the tumor on microscopic examination along with hemorrhage and fibroblastic proliferation. Karyorrhexis, karyolysis and calcification could be observed. In the viable portions of the tumor there

were mitotic figures. Metastatis was not observed. The uterus was enlarged due to endometrial hyperplasia; the uterine glands contained secretion and the blood vessels were hyperemic. The urinary bladder wall was thickened. Coagulation necrosis was seen in the renal tubules. The liver was congested. No visible lesion was found in the brain, gastrointestinal tract, pancreas or heart.

Group number 4 consisted of mice that received only corn oil along with the tumor. It can be seen in Plate III, Fig. 1, that little change occurred in the tumors of this group. Microscopically there were no significant pathologic changes in this group, other than those associated with the tumor. The tumor consisted of many viable areas characterized by numerous mitotic figures. Hemorrhage and areas of necrosis seen in many cases appeared to be the result of the tumor having outgrown its blood supply. Fibroblast proliferation was minimal. Metastases to the lungs were observed in a few instances. Lymphoid hyperplasia was seen in the spleen. Congestion was noted in the liver and lungs. No lesions were found in the brain, gastrointestinal tract, pancreas, heart muscle or uterus.

The mice comprising group 5 served as tumor-injected controls (Plate III, Fig. 2). The tumors in this group of mice had many viable areas with numerous mitotic figures and anaplastic cells. Occasionally areas of hemorrhage and necrosis were also observed that were due to the rapid proliferation of the tumor cells beyond their nutrition. Metastases were seen in the lungs. Infiltration of the rib cage was the rule in practically every case. Lymphoid hyperplasia was seen in the spleen; in many cases splenomegaly was evident on gross examination. Congestion was found in the liver and kidneys. No evidence of hyperplasia was found in either the uteri or

urinary bladders. No lesions were found in the gastrointestinal tract, pancreas, heart or brain.

PATHOGENESIS STUDY

1-3 days: At the site of tumor inoculation a mass of tumor cells was demonstrable microscopically intermingled with numerous inflammatory elements (Plate X, Fig. 1). The blood vessels adjacent to the area of the tumor cells were found to be hyperemic. Early fibroblast proliferation was also manifested by the host tissue. For the first two days no mitotic activity was noted in any of the neoplastic cells nor was there evidence of infiltration. Within the tumor mass, cell debris was found which consisted of disintegrated parts of the tumor brei.

Lymphoid hyperplasia was observed in the spleen; however, it was not very marked. The lungs were congested. Post mortem changes that were observed in the kidneys included coagulation necrosis of the proximal tubules and pyknosis of other parenchymal cell nuclei.

4-6 days: On the fourth day post-tumor-inoculation the mice received the first injection of the DEP-DA compound. By this time the tumor cells had started proliferating as was evidenced by mitosis and infiltration into the subcutis (Plate X, Fig. 2). There were numerous small blood vessels in the tumor mass indicating that the tumor had established itself in the host tissues. Adjacent to the tumor the blood vessels were hyperemic and there was fibroblast proliferation. By the sixth day the tumor was found to contain necrotic areas in which pyknosis was prominent.

The lungs were congested. Postmortem changes were observed in the kidneys as evidenced by coagulation necrosis and pyknosis; bacteria were

found intravascularly in one instance. Lymphoid hyperplasia was detected in the spleen. The uteri were found enlarged on gross examination; microscopically endometrial hyperplasia was observed. The urinary bladder was thickened due to an increase in the depth of the lamina propria and submucosa and in one instance there was squamous metaplasia.

7-9 days: Extensive areas of necrosis were found in the tumor which were characterized by pyknosis, karyorrhexis and karyolysis (Plate XI, Figs. 1 and 2). In the viable portions of the tumor the mitotic figures were reduced in number. There were no areas of hemorrhage. At the periphery of the tumor mass there was a slight reaction characterized by hyperemic blood vessels and fibroblast proliferation.

The lungs were congested and occasionally there was interstitial thickening. Lymphoid hyperplasia was noted in the spleen. Congestion was seen in the liver. Endometrial hyperplasia and active glandular secretion was found in the uteri. The pathologic changes in the kidney were those of post mortem autolysis.

10-12 days: Many necrotic areas were found in the tumor which had infiltrated into the subcutis and cutaneous muscle. Proliferation of fibroblasts was seen adjacent to the tumor. Mitotic figures were noticeably reduced in the viable areas of the tumor.

The lungs were congested and showed interstitial thickening. The liver was congested and in one instance micro-abscesses were found. Endometrial hyperplasia was a consistent lesion in the uterus. The urinary bladder was thickened due to increased lamina propria and submucosa. In a few instances squamous metaplasia was also observed. The kidney showed post mortem changes.

13-15 days: The tumor contained large areas of necrosis and hemorrhage.

There were also signs of calcification in a few instances but this was not prominent. Mitotic figures were markedly reduced in the viable portions of the tumor.

Lymphoid hyperplasia was observed in the spleen. The urinary bladder was thickened and occasionally revealed squamous metaplasia. The uterus was enlarged due to endometrial hyperplasia. The kidneys showed post mortem changes.

16-26 days: Extensive areas of hemorrhage and necrosis were found in the tumor. Calcification was also a prominent feature in the degenerating tumor cells. On the seventeenth, eighteenth and nineteenth day small metastatic tumor foci were observed in the lungs. This was rather contrary to expectations since metastases were not observed in the tumor inhibition studies with the higher dose of the DEP-DA.

The urinary bladder was thickened and occasionally squamous metaplasia was noted. Lymphoid hyperplasia was observed in the spleen. The uterus was enlarged due to endometrial hyperplasia.

DISCUSSION

Hormone therapy with estrogens in patients with breast cancer has been gradually discontinued because of undesirable side effects such as uterine bleeding, incontinence and hypercalcemia; it has become essential that for a related compound to be useful it should, in addition to inhibiting cancer, have no estrogenic activity. Knoche and Gawienowski (1962) proposed investigation of 3,6-derivatives of 9,10-diethyl phenanthrene derived from diethylstilbestrol and its derivatives by ultraviolet irradiation. Such a compound, 3,6-diacetate of 9,10-diethyl phenanthrene (DEP-DA) was synthesized by

ultraviolet irradiation of diethylstilbestrol diacetate (DES-DA) (Waring, unpublished data). The purpose of the present study was to investigate both the antitumor activity and estrogenic activity of DEP-DA and compare these actions with those of the parent compound, DES-DA.

Results of toxicity studies revealed that the new compound was estrogenic and that its action upon the uterus was quite marked. The uteri of the mice were enlarged on gross examination and histologic examination confirmed the presence of endometrial hyperplasia. Another organ often involved was the urinary bladder; the wall was thickened due to the increased lamina propria and submucosa. Occasionally squamous metaplasia of the urinary bladder epithelium was noted. These histopathologic changes were attributed to the estrogenic activity of the DEP-DA. The findings were much like those of Angrist et al. (1960) who observed squamous metaplasia in the urinary bladder following prolonged estrogen administration associated with a study on avitaminoses.

In the toxicity studies on the parent compound, DES-DA, the histopathologic findings were similar, but more marked. On gross examination the uteri were enlarged. Microscopically the lesions consisted of endometrial hyperplasia and squamous metaplasia; the latter change was consistent in all experimental mice in this group.

The antitumor activity of DEP-DA was characterized histologically by extensive areas of necrosis in the tumor and a decreased number of mitotic figures. Pyknosis, karyorrhexis, karyolysis and calcification were observed in the tumor cells. The above findings were in agreement with Peters et al. (1946) whose observations on damage to Sarcoma 37 with cephalin hydrochloride were similarly based on decreased mitotic activity of the tumor cells and

necrosis. It was observed that the most striking antitumor effect of the phenanthrene derivative in the present study was its capacity to arrest the mitotic activity in the tumor cells; these findings coincided with the observations of MacCardle and Downing (1947) with a colchicine compound and Donnelly et al. (1958) with mixed bacterial toxins used against Sarcoma 37.

In contrast with the findings of Finkelstein et al. (1951), who observed that multiple doses of 8-azaguanine produced reversible damage to Sarcoma 37 since typical morphology of the tumor cells was seen to return on cessation of therapy, the DEP-DA apparently caused a more complete destruction of the tumor cells for they did not show any tendency to recover normal morphology following treatment. Necrosis of the tumor cells with cellular disintegration and calcification were observed along with fibroblast proliferation adjacent to the areas of necrosis. The results obtained in the present study were in agreement with Brues et al. (1937) and Turner (1939) who observed that the phenanthrene nucleus might be responsible for the antitumor activity of colchicine compounds. The results were also in agreement with Field et al. (1959) who pointed out that all active phenanthrene compounds had substituents either in the 3-position or mesoposition and often in both.

In the comparative tumor studies in which DES-DA was used, antitumor activity was characterized by areas of necrosis and reduced numbers of mitotic figures in the tumor cells. Since the compounds had similar configuration, this duplication of effect was not considered unusual. It should be noted that thickening of the uterine wall due to endometrial hyperplasia was consistently observed but without a single instance of squamous metaplasia as had occurred in the toxicity study. No explanation was found for this. The wall of the urinary bladder was similarly thickened due to increased lamina

propria and submucosa with a few instances of squamous metaplasia; the findings were not unlike those of the toxicity study on DES-DA. Gardner (1941) and other workers observed endometrial hyperplasia on prolonged administration of estrogens but none reported squamous metaplasia in the uterus. However, Bittner (1941), Allen et al. (1941), Pan et al. (1948), Svaboda (1961), and other workers induced mammary tumors and carcinomas of the cervix uteri by prolonged administration of estrogens in mice.

The most significant change observed in the pathogenesis studies in which the mice were treated with a higher dose of DEP-DA, was that the tumor developed necrotic areas as early as the sixth day, i.e., three days after the first injection of the drug. In contrast the untreated tumors took 10 days or more to develop necrotic areas in the normal course of their growth.

The 3,6-diacetate of 9,10-diethyl phenanthrene was shown to possess antitumor activity principally evidenced by arresting mitoses in the tumor cells. In spite of its estrogenic activity the new compound may prove to be valuable on further investigation.

SUMMARY

Studies are reported on the effect of a newly synthesized compound, 3,6-diacetate of 9,10-diethyl phenanthrene obtained by ultraviolet irradiation of diethylstilbestrol diacetate, against Sarcoma 37 in tumor susceptible mice of the C_3H/Hej strain. The compound is shown to possess a rather pronounced antitumor activity which is demonstrated microscopically by extensive areas of necrosis and hemorrhage and reduced numbers of mitotic figures in the tumor cells; antitumor activity is characterized primarily by the arresting of mitoses. The compound is also seen to possess estrogenic activity

evidenced microscopically by endometrial hyperplasia in all experimental mice with a few instances of squamous metaplasia in both the uterus and the urinary bladder.

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APPENDIX

EXPLANATION OF PLATE I

Fig. 1. Mouse from Group 1 of the tumor inhibition study which received DEP-DA at a rate of 45 mg./kg. Front view to show hemorrhage and crateriform effect. Picture taken on fourteenth day post tumor inoculation.

Fig. 2. Mouse from Group 1 of the tumor inhibition study, which received DEP-DA at a rate of 45 mg./kg. Picture taken on eighteenth day post tumor inoculation to show the height of the tumor.

PLATE I

Fig. 1

Fig. 2

EXPLANATION OF PLATE II

Fig. 1. Mouse from Group 2 of the tumor inhibition study, which received DEP-DA at a rate of 90 mg./kg. Picture taken on eighteenth day post tumor inoculation to show height of the tumor.

Fig. 2. Mouse from Group 3 of the tumor inhibition study, which received DES-DA at a rate of 36 mg./kg. Picture taken on eighteenth day post tumor inoculation to show the size and condition of the tumor for comparison with those that received DEP-DA.

PLATE II

Fig. 1

Fig. 2

EXPLANATION OF PLATE III

Fig. 1. Mouse from Group 4 of the tumor inhibition study, which received 0.2 ml. of corn oil only. Picture taken on eighteenth day post tumor inoculation to show the height and growth of the tumor.

Fig. 2. Mouse from Group 5 of the tumor inhibition study, which received only the tumor. Picture taken on eighteenth day post tumor inoculation to show the growth and condition of the tumor.

PLATE III

Fig. 1

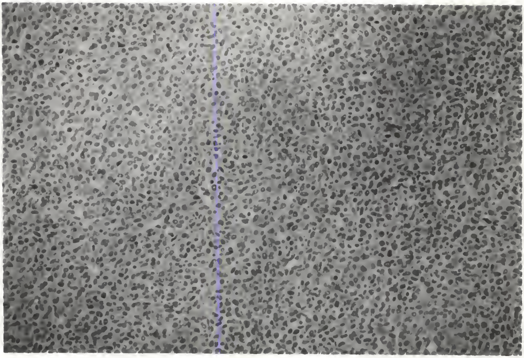
Fig. 2

EXPLANATION OF PLATE IV

Fig. 1. Photomicrograph of a tumor from C_3H/Hej control mouse which received no compound. Solid mass of cells interspersed with small amounts of stroma. The tumor is seven days old. Hematoxylin and eosin; X 125.

Fig. 2. Photomicrographs of the tumor from Group 5 of the tumor inhibition study which received only tumor. Mitotic figures are numerous. Tumor cells are pleomorphic. Hematoxylin and eosin; X 500.

PLATE IV



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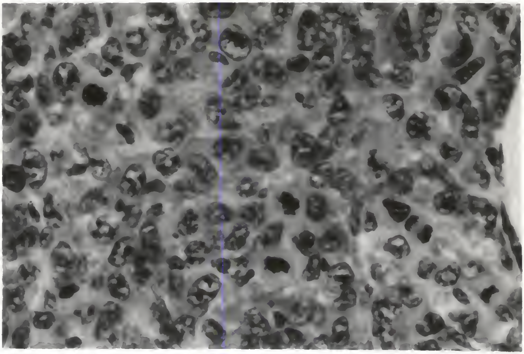


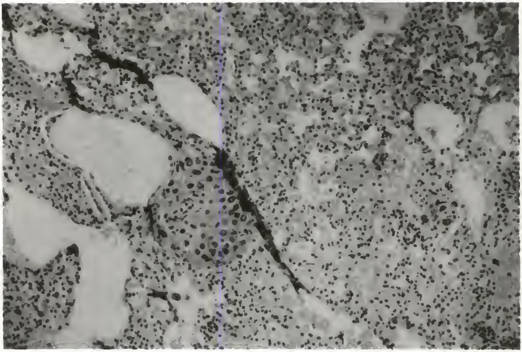
Fig. 2

EXPLANATION OF PLATE V

Fig. 1. Photomicrograph of a lung of a mouse from Group 5 of the tumor inhibition study which received only tumor. Foci of metastasis by tumor cells. Hematoxylin and eosin; X 125.

Fig. 2. Photomicrograph of a lung of a mouse from Group 5 of the tumor inhibition study which received only tumor. Metastasis in the lung. Hematoxylin and eosin; X 500.

PLATE V



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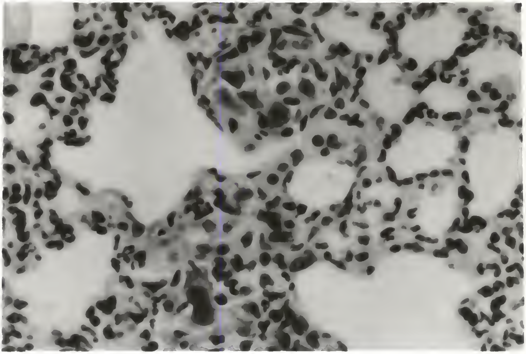


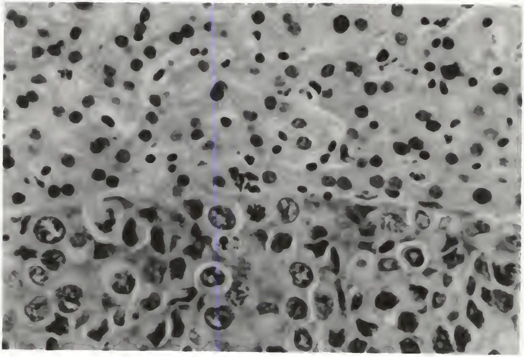
Fig. 2

EXPLANATION OF PLATE VI

Fig. 1. Photomicrograph of the liver of a mouse from tumor control Group 5 of the tumor inhibition study which received only tumor. Extension of the tumor into liver. Upper half is liver; lower half is tumor. Hexatoxylin and eosin; X 125.

Fig. 2. Photomicrograph of the liver of a mouse from tumor control Group 5 of the tumor inhibition study which received only tumor. Tumor cells in the liver by metastasis. Hematoxylin and eosin; X 500.

PLATE VI



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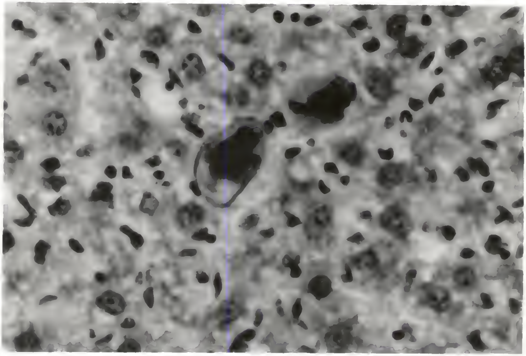


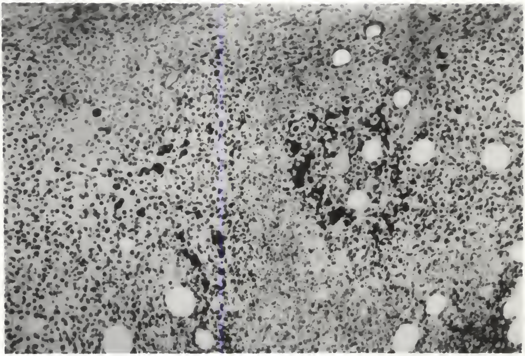
Fig. 2

EXPLANATION OF PLATE VII

Fig. 1. Photomicrograph of a tumor from Group 2 of the tumor inhibition study which received DEP-DA at a rate of 90 mg./kg. Calcification in the degenerating tumor cells. Hematoxylin and eosin; X 125.

Fig. 2. Photomicrograph of a tumor removed from C_3H/Hej control mouse after seven days of growth. Reticular fibres in the viable areas of the tumor. Necrotic area is devoid of reticular fibres. Wilder's silver stain; X 125.

PLATE VII



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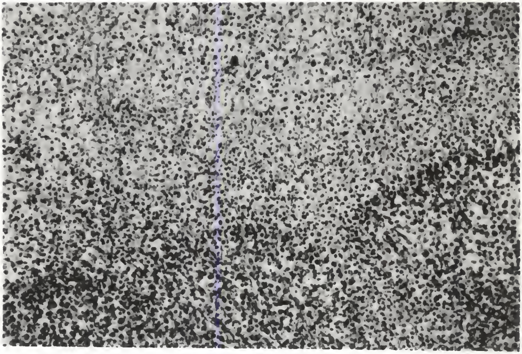


Fig. 2

EXPLANATION OF PLATE VIII

Fig. 1. Photomicrograph of a uterus of a mouse in the toxicity study which received DEP-DA at a rate of 46.6 mg./kg. Endometrial hyperplasia. Hematoxylin and eosin; X 125.

Fig. 2. Photomicrograph of a uterus of a mouse from the toxicity study which received DEP-DA at a rate of 96.5 mg./kg. Endometrial hyperplasia. Hematoxylin and eosin; X 125.

PLATE VIII

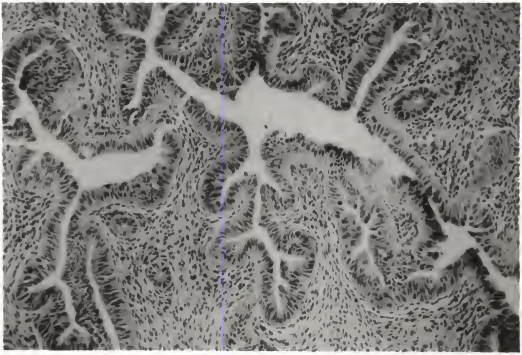


Fig. 1

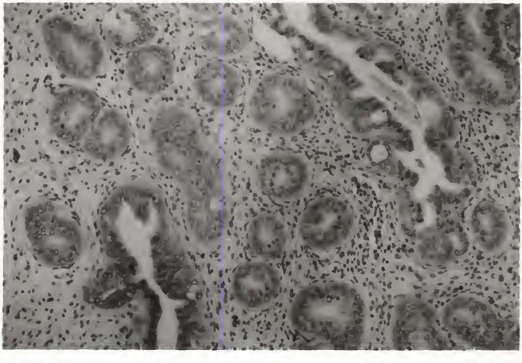


Fig. 2

EXPLANATION OF PLATE IX

Fig. 1. Photomicrograph of a urinary bladder of a mouse from Group 2 of the tumor inhibition study which received DEP-DA at a rate of 90 mg./kg. Epithelium has a tendency towards squamous metaplasia. Hematoxylin and eosin; X 125.

Fig. 2. Photomicrograph of a uterus of a mouse from the toxicity study which received DES-DA at a rate of 45.8 mg./kg. Squamous metaplasia of the epithelium. Hematoxylin and eosin; X 125.

PLATE IX

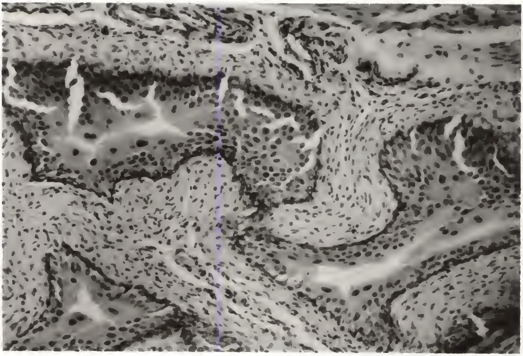


Fig. 1

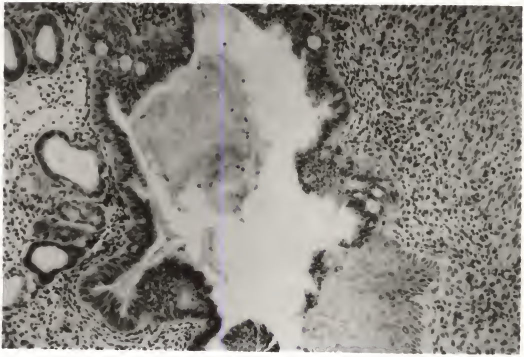


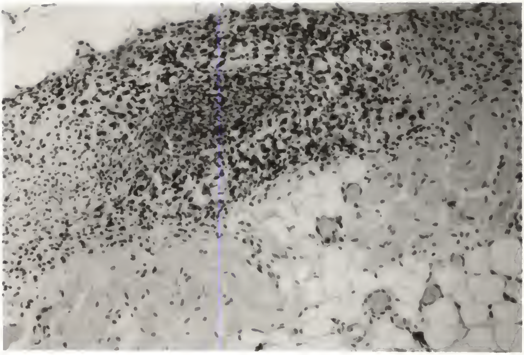
Fig. 2

EXPLANATION OF PLATE X

Fig. 1. Photomicrographs of a tumor from second day of the pathogenesis study. The site is that of tumor inoculation. Tumor cells are intermingled with inflammatory cells. Hematoxylin and eosin; X 125.

Fig. 2. Photomicrograph of a tumor from fourth day of the pathogenesis study. Tumor cells are proliferating and infiltrating into the subcutis. Hematoxylin and eosin; X 125.

PLATE X



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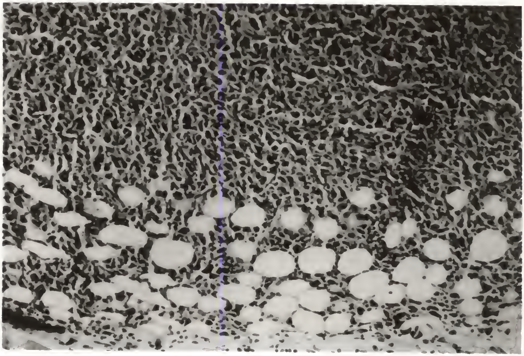

Fig. 2

EXPLANATION OF PLATE XI

Fig. 1. Photomicrograph of a tumor from Group 2 of the tumor inhibition study which received DEP-DA at a rate of 90 mg./kg. Necrotic areas evidenced by pyknosis, karyorrhexis and karyolysis. Viable areas are also seen. Hematoxylin and eosin; X 125.

Fig. 2. Photomicrograph of a tumor from Group 2 of the tumor inhibition study which received DEP-DA at a rate of 90 mg./kg. Necrotic areas evidenced by pyknosis, karyorrhexis and karyolysis. Viable areas are also seen. Hematoxylin and eosin; X 125.

PLATE XI

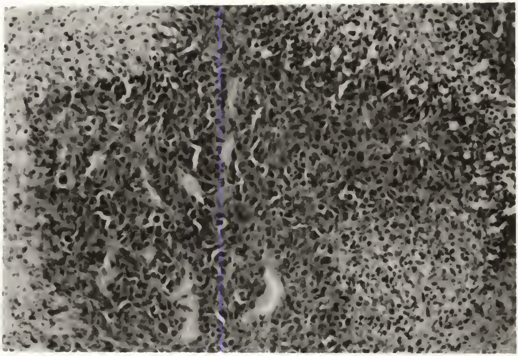

Fig. 1

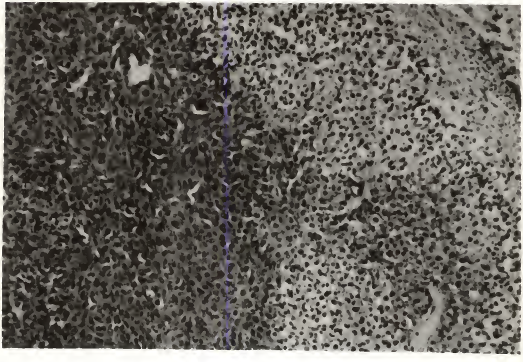

Fig. 2

AN HISTOPATHOLOGIC STUDY OF THE EFFECTS OF A SUBSTITUTED PHENANTHRENE DERIVATIVE ON SARCOMA 37 IN MICE

by

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AN ABSTRACT OF A MASTER'S THESIS

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requirements for the degree

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1966

Treatment and control of cancer by surgical intervention and irradiation techniques has been well established. The search for chemotherapeutic agents with the same efficacy has resulted in the finding of very few drugs which have both antitumor properties and minimal side effects. The present study represents a preliminary screening of another potential chemotherapeutic agent.

The newly synthesized compound, 3,6-diacetate of 9,10-diethyl phenanthrene (DEP-DA), was studied for side effects and for its antitumor activity on Sarcoma 37 in tumor-susceptible C_3H/Hej mice. The compound (DEP-DA) was prepared by ultraviolet irradiation of diethylstilbestrol diacetate (DES-DA), which also was included in the study for comparative purposes.

Preliminary studies were made with corn oil, olive oil and saline which were the carriers in which the drug would later be administered. Each of three groups of mice were injected with one of the above carriers and similar groups were kept as controls.

Toxicity studies were performed on four groups of C_3H/Hej female mice. The first and second group of mice received DEP-DA at a rate of 46.6 mg./kg. and 96.5 mg./kg. respectively. The third group received DES-DA at a rate of 45.8 mg./kg. The mice of the fourth group received 0.2 ml. of corn oil only. Five injections were made at daily intervals. The mice were sacrificed seven days after the last injection for gross and microscopic evaluation.

Sarcoma 37 was obtained from the Jackson Laboratory, Bar Harbour, Maine, in DBA/1J mice and immediately transplanted into C_3H/Hej female mice which were used for tumor inhibition studies. The mice were weighed in groups to determine the dosage of the compound to be tested. The first and second groups of mice received DEP-DA at a rate of 45 mg./kg. and 90 mg./kg.

respectively. The third group of mice received DES-DA at a rate of 36.6 mg./kg. The mice of the fourth group received 0.2 ml. of corn oil only and the fifth group received no injection other than the tumor.

In the pathogenesis studies mice received the drug from the fourth day post tumor injection. The mice were sacrificed at daily intervals for 26 days.

Mice from the toxicity, tumor inhibition and pathogenesis studies were preserved in 10% formalin and the tissues were collected for histopathologic examination after routine staining with hematoxylin and eosin. Special stains were employed where indicated.

Preliminary studies with the carriers revealed microscopically a granulomatous reaction in mice that received corn oil; however, this oil was used in subsequent studies because the DEP-DA and DES-DA were more soluble in it.

In the toxicity studies the uteri were found to be enlarged both in those mice receiving DEP-DA and those which received DES-DA. With the former drug only endometrial hyperplasia was noted. With the latter compound squamous metaplasia was observed in addition to the endometrial hyperplasia. The wall of the urinary bladder was thickened due to increased lamina propria and submucosa in both instances.

In the tumor inhibition studies extensive areas of necrosis were seen with DEP-DA, especially when employed at the higher level. Necrosis was microscopically evidenced by pyknosis, karyorrhexis, karyolysis and calcification. Mitotic figures were markedly reduced in the viable areas of the tumor. The DEP-DA was observed to act primarily by arresting mitoses. The DES-DA was found to cause comparable changes. Endometrial hyperplasia was

a consistent finding in these mice. The urinary bladder was very often grossly thickened; microscopically squamous metaplasia of the epithelium was observed in several instances.

The most significant change observed in the pathogenesis studies in which the mice were treated with a higher dose of DEP-DA, was that the tumor developed necrotic areas as early as the sixth day, i.e., three days after the first injection of the drug. In contrast the untreated tumors took 10 days or more to develop necrotic areas in the normal course of their growth.

The 3,6-diacetate of 9,10-diethyl phenanthrene was shown to possess antitumor activity principally evidenced by arresting mitoses in the tumor cells. In spite of its estrogenic activity the new compound may prove to be valuable on further investigations.